1-2015

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eCommons Citation

Crecelius, Anne R.; Kirby, Brett S.; and Dinenno, Frank A., "Intravascular ATP and the regulation of blood flow and oxygen delivery in humans" (2015). *Health and Sport Science Faculty Publications*. 56.
https://ecommons.udayton.edu/hss_fac_pub/56

Intravascular ATP and the regulation of blood flow and oxygen delivery in humans

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Character Count (with spaces): 42,124
Short Title: ATP in vascular control in humans

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Funding: NIH Grants: AG027150, HL087952, HL095573, AG022337, HL102720 (F.A.D.) and Colorado State University Monfort Professorship (F.A.D.)

Conflicts of Interest: None

Abstract (40-60 words): Regulation of vascular tone is a complex response that integrates multiple signals which allow for blood flow and oxygen supply to appropriately match oxygen demand. Here, we discuss the potential role of intravascular ATP as a primary factor in these responses and propose that deficient ATP release may contribute to impairments in vascular control exhibited in aged and diseased populations.

Summary (15-20 words): ATP is involved in vascular control during exercise and hypoxia and may explain impaired regulation in certain high-risk populations.

Key Words: blood flow, hypoxia, exercise, purine, hyperemia

INTRODUCTION

Appropriate matching of oxygen delivery to tissue metabolic demand occurs largely via adjustments in convective oxygen delivery, or more simply, changes in blood flow. In healthy humans, blood flow is linearly correlated with oxygen consumption in tissue beds such as cardiac and skeletal muscle and augmentation of oxygen demand (e.g. muscle contractions) is met with a synchronized increase in blood flow and oxygen supply (20). In the case of challenged oxygen supply, as in systemic hypoxia, where hemoglobin oxygen saturation is lower than normal, blood flow increases to normalize oxygen delivery (i.e. blood flow × arterial blood oxygen content) to the given demand (6,27,30). Accordingly, any oxygen mismatch evoked by either an acute elevation in oxygen demand, reduction in oxygen delivery, or an exacerbated combination of both stimuli, results in an elevation in tissue blood flow.

Absolute tissue blood flow is determined by perfusion pressure and arteriolar resistance. During physiological stresses, both determinants of blood flow are modulated and influential; however, it is vascular resistance that has greater impact since changes in vessel diameter are magnified to the 4th power (Poiseuille's law). Arteriolar vascular caliber is controlled by multiple input signals including sympathetic vasoconstrictor tone, circulating vasoactive hormones or neurotransmitters, transmural pressure, endothelial-derived substances and metabolic factors acting on the vessel extra- and intra-luminally. At the regional and microcirculatory level, a great deal of redundancy is observed with regard to regulating net vascular tone and this is often evidenced by the failure of pharmacological inhibition of one or more vasoactive factors to impair metabolic autoregulation (20).

Metabolic autoregulation has been the topic of many investigations in both animal and human models and the compilation of findings clearly demonstrates an extremely complex control of vascular tone. Specific vasoactive candidates important in the control of vascular tone during physiological stress are often framed against a number of criteria. First, regarding the endogenous molecule, it must be measureable, have inactivation mechanisms, and release should occur at the required location and be stimulated during the stress. Secondly, if the candidate molecule is administered exogenously, the vasoactive response should mimic that which occurs during the stress. Finally, inhibiting the vascular action of the molecule should be consistent with the proposed hypothesis of vascular regulation (12). Recent insights gained from our laboratory and others demonstrate that intravascular ATP is a candidate molecule that largely satisfies these criteria. Therefore, the purpose of this review is to present the most significant and recent data related to the role of intravascular ATP in vascular control of humans including the potential sources and stimuli for endogenous release and the signaling pathways underlying ATP's powerful vasoregulatory action. Finally, we present the postulate that changes in the regulation of intravascular ATP and associated vasomotor signaling may contribute to observed impairments in vascular control of aged or diseased humans during conditions of oxygen mismatch.

INTRAVASCULAR ATP IS MEASURABLE DURING EXERICSE AND HYPOXIA

In the late 1960's, Forrester and Lind reported an increase in venous plasma ATP concentrations during exercise in humans (13). Since that time, our laboratory and others have advanced understanding through investigations demonstrating an increase in plasma [ATP] during graded intensity whole-body or isolated limb models of exercise in humans (4,14,16,25,30). In general, the observed elevations in intravascular [ATP] are greatest in venous blood draining the active muscle and occur in an exercise intensity (and tissue oxygen consumption) dependent manner (Figure 1A-B). In regard to hypoxia, when humans are exposed to low-oxygen content air (~10% fractional inspired O_2; O_2 saturations ~80%), venous plasma [ATP] draining muscle is also elevated in young healthy humans (Figure 1C-D) (16,25,30). Along with increases occurring primarily in the venous circulation, the degradation of ATP via cell-surface ectonucleotideases appears to be rapid (half-life of intravascular ATP < 1 second) and thus it is likely that it is a local release of ATP within or in close proximity to the microcirculation that drives elevations in plasma [ATP] draining skeletal muscle during exercise and hypoxia (25,30).

It is important to note that careful consideration must be made towards reported absolute values of plasma [ATP] due to differences in technical measurement (luciferin-luciferase assay vs high performance liquid chromatography), sample location (e.g. intravascular microdialysis, large and small vessels) and processing (e.g. preservation or "stop" solutions, immediate vs delayed measurement) (17). These discrepant absolute values are apparent in the data reproduced in Figure 1. Also, in these *in vivo* human studies, samples were obtained from vessels either up- (arterial) or down- (venous) stream of the skeletal muscle microcirculation where intravascular [ATP] would be greatest and impact vascular tone. Despite these considerations, within a given study, it is well-established that muscle contractions and systemic hypoxia increase venous plasma [ATP] draining skeletal muscle in humans (4,13-16,25,30).

FROM WHERE COULD ATP BE RELEASED DURING EXERCISE AND HYPOXIA?

In order for intravascular ATP to have a role in vascular control during mismatches in oxygen delivery and demand, cellular sources of ATP must be able to release ATP during these stimuli at the required location of the microcirculation. Potential candidates for the source(s) of increased intravascular [ATP] during exercise and hypoxia are discussed below.

Extravascular sources: sympathetic nerves and skeletal muscle cells

During systemic hypoxia and moderate to high-intensity exercise, sympathetic nervous system activity is increased (20,27). ATP can be co-released from sympathetic nerves along with norepinephrine and thus investigators have questioned whether the nerves may contribute to the observed increased plasma [ATP] under these conditions. Additionally, interstitial [ATP] increases during exercise and skeletal muscle itself has also been proposed to be a potential source of plasma ATP during muscle contractions (28). However, given the size of ATP molecules, its rapid degradation by ectonucleotidases located on cell surfaces, as well as previous findings that intravascular infusion of exogenous ATP does not elevate interstitial concentrations suggests vascular smooth muscle and endothelial cells may provide an effective barrier that prevents nerve- and/or muscle-released ATP from reaching the intravascular space (28).

Recently, we determined whether acute elevations in sympathetic nervous system activity increases venous plasma [ATP] draining skeletal muscle in humans. Lower body negative pressure to elicit baroreflex-mediated activation of the sympathetic nervous system failed to increase venous plasma [ATP] both at rest (Figure 2A) and during exercise (Figure 2B) (22). Regarding skeletal muscle as a potential source, muscle contractions fail to independently increase [ATP] when blood flow is occluded (Figures 2C and 2D). Thus, the collective data indicate that sympathetic nerves and skeletal muscle cells likely do not contribute to the observed increases in plasma [ATP] during exercise or other sympathoexcitatory conditions such as systemic hypoxia.

Intravascular and vascular sources: blood cells and endothelial cells

Manipulation of forearm blood flow in the aforementioned study not only allowed for determination of whether the sympathetic nerves and/or skeletal muscle may be a source of ATP but also provided insight as to whether perfusion itself, and therefore supply of erythrocytes, other blood cells, and shear stress along endothelial cells is obligatory to observe increased venous plasma [ATP] draining skeletal muscle during exercise (22). When perfusion to actively contracting muscle is occluded, plasma [ATP] levels decline to resting values (Figure 2C). Additionally, when blood flow to a resting tissue is occluded and then muscle contractions commence, plasma [ATP] does not increase (Figure 2D). Taken together, our findings suggests the source of increased plasma [ATP] during exercise is dependent on perfusion and thus from cells within or in contact with the blood. In this context, isolated erythrocytes release significant amounts of ATP in response to a variety of stimuli which we discuss in further detail in the following section (1,11). Similarly, vascular endothelial cells, the monolayer of epithelial cells capable of releasing a variety of vasoactive substances in isolation, can release ATP under similar conditions (2). While the specific cell source of increased plasma [ATP] draining skeletal muscle remains somewhat elusive, collective evidence suggests it is within or in contact with the intravascular space.

WHAT STIMULATES THE RELEASE OF ATP DURING EXERCISE AND HYPOXIA?

In order for ATP to be a candidate molecule involved in the regulation of vascular tone during muscle contractions or systemic hypoxia, specific stimuli for ATP release must be present during these physiological stresses. To date, a number of stimuli have been proposed to increase intravascular [ATP] under these conditions.

Changes in blood milieu

Repeated muscle contractions evoke an increase in metabolism, which results in greater oxygen consumption, the production of CO_2, and acidosis (4,6,28). Classic physiology studies manipulating hemoglobin concentrations demonstrated that muscle blood flow is more directly dependent on changes in oxygen content rather than changes in the partial pressure of oxygen (PO_2) (31). In this context, it is important to note both *in vitro* data in whole blood samples (Figure 2E) (18) and plasma measurements of [ATP] from humans (Figure 2F) (16) show a strong relationship between increased ATP release and hemoglobin deoxygenation. Although declines in PO_2 that accompany exercise and hypoxia may stimulate endothelial cell release of ATP independent of erythrocytes (2), there is evidence demonstrating that isolated resistance vessels fail to dilate in response to hypoxia without the presence of erythrocytes (8), thus it is unlikely that the endothelium is the primary site of ATP release.

In addition to deoxygenation that occurs with muscle contractions or systemic hypoxia exposure, changes in pH, particularly acidosis can also stimulate ATP release from red blood cells (11). Further, as a result of repeated muscle contractions, increases in blood temperature can occur and this may also contribute to ATP release during exercise (21). The specific ATP release pathways from erythrocytes and other cell sources and the various stimuli for these processes continue to be a topic of interest. Taken together, the combined local metabolic milieu resultant from exercise or hypoxia is a stimulus for increased ATP release from intravascular sources and would appropriately increase with greater exercise intensity or duration of these conditions of oxygen mismatch, as do ATP levels (Figure 1A-B).

Mechanical stresses

In addition to the changes in metabolic milieu associated with exercise that have been shown to stimulate ATP release, mechanical stimuli during muscle contraction have also been associated with increased ATP release. It is well known that as erythrocytes traverse the microcirculation they undergo mechanical deformation, and this has experimentally been shown to stimulate ATP release (11). As skeletal muscles contract, the elevation in extravascular pressure causes compression or distortion of the resistance vessels, thus exposing the erythrocytes to even greater mechanical stress during exercise. Endothelial cells also experience increased mechanical stimulation during exercise in the form of greater shear stress due to elevated blood flow and mechanical distortion as a result of contracting tissue. Studies *in vitro* demonstrate these factors increase endothelial cell ATP release (2). Along these lines, we and others have shown mechanical stimulation to mimic the compressive forces of a muscle contraction (via rhythmic inflation and deflation of a blood pressure cuff) increases venous plasma [ATP] (4,15). It should be noted that *in vivo* human models cannot determine the cell-specific source of ATP in these conditions as it is not possible to selectively cause mechanical distortion of erythrocytes or endothelial cells. However, when vasodilators are infused into the brachial or femoral artery and increase blood flow and shear stress along endothelial cells without changing oxygenation, [ATP] does not increase in the venous effluent (25,30). Thus, it does not appear that shear-mediated endothelial cell release largely contributes to the increase in plasma [ATP] draining skeletal muscle observed during exercise and/or hypoxia.

WHAT ARE THE VASOMOTOR ACTIONS OF EXOGENOUS ATP AND DO THESE MIMIC THOSE OF EXERCISE AND HYPOXIA?

Historically, when attempting to discern the role for a given substance or signaling cascade in vascular control, pharmacological antagonists or physiological maneuvers are utilized to inhibit the source or action of the substance in question and determine the possible impact on the regulation being studied, for instance exercise hyperemia. One of the significant challenges that we and others have faced in our investigations of ATP is the lack of an appropriate pharmacological antagonist to inhibit ATP binding to its respective purinergic receptors (P_2). Even in animal models, specific pharmacology is limited and thus the data in support of our overall hypothesis of vascular regulation are derived from physiological and experimental manipulations of the previously discussed stimuli, as well as the unique signaling pathways and vasomotor properties of ATP as described below.

Potent Vasodilation

The potential role for extracellular ATP as a vasoactive molecule in humans was initially described in the mid-20th century when observations of increased blood flow were made following exogenous ATP intra-arterial infusion (9). As compared to other purine compounds such as adenosine, the potency of ATP is robust, causing significant dilation that mimics levels achieved during maximal exercise (Figure 3A) (32).

Modulation of Sympathetic Vasoconstriction

In addition to the vasodilator properties of ATP, this nucleotide is also able to modulate post-junctional sympathetically-mediated vasoconstriction, a property unique amongst exogenous vasodilator substances in humans (Figure 3B) (24,26,32). Our working hypothesis is that ATP binds to P_2 receptors on the endothelium and this leads to hyperpolarization of endothelial and vascular smooth muscle cells which in turn, limits sympathetic vasoconstriction. Importantly, the ability to limit post-junctional sympathetic vasoconstriction is a significant phenomenon that occurs in actively contracting skeletal muscle and is known as 'functional sympatholysis'. Given the profound vasodilator capacity of the skeletal muscle vasculature, vasoconstriction even within the active muscle is needed in order to prevent a decline in total peripheral resistance and thus maintain arterial blood pressure. In this manner, functional sympatholysis permits increased blood flow and oxygen delivery to the active tissue in order to support increased metabolism. It is therefore critical that intravascular ATP possesses dual vasomotor properties in that it can facilitate hyperemia by causing direct vasodilation during exercise and also act to limit the amount of sympathetic vasoconstriction, thereby preserving adequate blood flow to the active tissue during conditions of sympathoexcitation.

DOES INHIBITING ATP SIGNALLING ALIGN WITH THE HYPOTHESIS?

Early *in vitro* data demonstrated that ATP stimulated vasodilation via an endothelium-dependent mechanism; however, downstream obligatory signaling pathways in humans remained uncertain for some time. In humans, a variety of investigations explored whether the endothelial-derived autocoids nitric oxide and prostaglandins explained ATP vasodilation with equivocal results being found, even in our own laboratory depending on the timing of inhibition and method of blood flow measurement (5). Upon critical review of the collective data, it appears that up to 20% of the vasodilation stimulated by ATP may be due to NO and PGs in humans.

In contrast, intra-arterial infusion of barium chloride to inhibit inwardly-rectifying potassium (K_{IR}) channels reduces ATP-mediated dilation ~50% (Figure 4A) (3). Activation of K_{IR} channels is understood to directly hyperpolarize vascular cells (endothelial and/or smooth muscle) (10) as well as amplify hyperpolarization signals originating from adjacent cells (19). Importantly, vascular hyperpolarization is the essential underpinning for conducted vasodilation, or specifically, the ability for electrical signals to rapidly spread throughout the vasculature and cause profound vasodilation (33). Taken together, these data are consistent with the hypothesis that intravascular ATP and the ensuing signaling cascade is a robust regulator of vascular tone.

Recently, we have inhibited K_{IR} channels during muscle contractions in humans (7). In our model of forearm exercise, there is no impact on hyperemia during steady-state exercise when the synthesis of NO and PGs are antagonized; however, inhibiting K_{IR} channels significantly reduces exercise hyperemia by ~30% (Figure 4B). The magnitude of this effect is profound and to date represents the largest impact on forearm exercise hyperemia via pharmacological antagonists of single or multiple vasodilator pathways (20).

Consistent with the significant impact on exercise hyperemia of inhibiting K_{IR} channels, hyperpolarization and resultant conducted dilation may be crucial to the robust vasodilation and modulation of sympathetic vasoconstriction that occurs in the microvasculature during muscle contraction (33). Taken together, activation of K_{IR} channels resulting in hyperpolarization of the vasculature appears to be crucial to vasomotor regulation during exercise and importantly, ATP signals via this mechanism.

Regarding hypoxic vasodilation, we have previously explained local mechanisms of hypoxic vasodilation in humans via inhibition of NO and PGs (27). Our current studies are attempting to determine whether hyperpolarizing pathways are also involved in the hypoxic response, particularly that which occurs when hypoxia is combined with exercise, which cannot be completely attributed to NO derived from nitric oxide synthase and PGs (6). Given that some data indicate a portion of ATP-mediated vasodilation can signal via NO and PGs, it still remains a possibility that ATP may mediate the rise in blood flow that allows for perfusion matching in circumstances of decreased oxygen supply.

INTRAVASCULAR ATP IN HUMANS OF HIGH DISEASE RISK

Thus far, we have built support for the hypothesis that ATP is involved in vascular control during mismatches of oxygen delivery and demand in young healthy humans. As depicted in Figure 5A, exercise increases tissue oxygen demand whereas hypoxia decreases tissue oxygen supply. Additionally, during exercise CO_2 increases and acidosis occurs. These stimuli, along with exercise-induced mechanical factors and increases in blood temperature can serve as stimuli for ATP release from intravascular cell sources. Circulating extracellular ATP then signals for increased vasodilation and blunts sympathetically-mediated vasoconstriction. Both actions increase red cell supply and therefore oxygen delivery to the tissue in need. The net hyperemic response works in a negative feedback manner to limit the original oxygen deficit and thus a steady-state homeostasis is reached until exercise or hypoxic exposure changes or ceases.

Investigations to date have largely focused on the role of extracellular ATP in vascular control of young healthy humans. As such, studies in older individuals (>60 years of age) and patient populations to determine vasomotor responsiveness to ATP and/or the level of extracellular ATP release into the bloodstream are limited, despite these populations being at an increased risk for cardiovascular morbidity and mortality. Nevertheless, evidence from our laboratory demonstrates an intact vasodilator responsiveness and sympatholytic capacity of exogenous ATP at rest in the forearm of aged humans despite the presence of classic 'endothelial dysfunction' (23,24). Similarly, in the leg vasculature of diabetic humans, both dilator responsiveness and sympatholytic capacity of exogenous ATP appear to be largely preserved (35). It should be noted that preserved vasodilator capacity to ATP with advanced age is not a universal finding (29), however at present we interpret the existing data to indicate that the net vasomotor responses to intravascular ATP remains generally intact with age and in certain disease populations.

With regard to measuring endogenous plasma [ATP] in high-risk humans at rest or during mismatched oxygen supply and demand conditions, very few studies have been published. In a recent study from our laboratory, we demonstrated that older healthy humans have reduced blood flow due to impaired local vasodilation during both graded handgrip exercise and systemic hypoxia relative to young adults, and this was associated with impaired increases in plasma [ATP] with age (Figure 6) (25). Further, we demonstrated that elevated ATP catabolism during the stimulus was not responsible for the low plasma [ATP] values in older adults, but rather isolated erythrocytes from this population fail to release a significant quantity of ATP in response to hemoglobin deoxygenation at levels observed during muscle contraction and systemic hypoxia (Figure 7A). Similarly, erythrocytes obtained from type II diabetic patients fail to release ATP in response to deoxygenation (Figure 7B) (34). Impaired erythrocyte release of ATP has also been observed in pulmonary hypertension, cystic fibrosis, and sickle cell patients, all conditions typically associated with dysfunctional vasomotor control at rest and presumably during oxygen mismatch, although the latter has not been completely determined (11).

Collectively, we propose the overall scheme (Figure 5B) that in older and diseased individuals, the failure to adequately increase intravascular [ATP] partially explains attenuated exercise and hypoxic vasodilation (24,25) as well as an impaired ability to modulate sympathetic vasoconstriction (24) in these populations. Whereas in young healthy adults ATP functions to assist matching of oxygen delivery to demand in a homeostatic function, this feedback control system is defective in aged or diseased individuals and fails to regulate blood flow and oxygen supply, thus allowing the oxygen mismatch to persist or become exacerbated.

SUMMARY

The regulation of muscle blood flow during conditions of mismatched oxygen delivery and demand is a complex interaction of a variety of factors including neuronal signals and local chemical and mechanical stimuli. Here, we have reviewed the recent literature that suggests ATP may be an important local signaling molecule in this regard as it fits the aforementioned criteria for likely candidates of vascular control during physiological stress. First, exercise and hypoxia evoke measureable elevations in skeletal muscle plasma ATP. This ATP is expected to be of intravascular origin and is appropriately located to increase blood flow and oxygen delivery to tissues of metabolic need. While not extensively discussed in this review, several inactivation mechanisms are in place to regulate plasma ATP concentrations (e.g. ectonucleotidases) and thus finely control the resulting vasoactive action. Second, exogenous administration of ATP mimics the predicted responses of exercise specifically in terms of robust vasodilation and the ability to modulate sympathetic vasoconstriction. Third, while specific and selective inhibition of intravascular ATP and the concomitant vascular signaling during exercise remains difficult, pharmacology known to significantly blunt ATP-induced hyperemia in quiescent muscle correspondingly results in profound attenuation of exercise hyperemia in humans (~30%). Further, we propose that due to their impaired ability to release ATP, older healthy and diseased humans exhibit compromised vascular control during cases of mismatches in oxygenation and in turn this leads to further impairments in oxygen delivery. Future research should be aimed at determining therapeutic interventions to improve ATP release and increase intravascular ATP in these at-risk populations. The resulting normalized vascular control may mitigate the current elevated risk of cardiovascular mortality and acute cardiovascular events in these populations.

ACKNOWLEDGEMENTS

We would like to thank the current and past members of the Human Cardiovascular Physiology Laboratory at Colorado State University for their efforts on these projects as well as the subjects who volunteered to participate. We would also like to thank the countless investigators who have contributed to the ideas presented here. Due to limitations on references we are unable to cite many important contributions (particularly studies in experimental animals) and have cited other great reviews on this topic as opposed to the original reference, and for this we apologize.

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FIGURE LEGENDS

Figure 1. Increased venous plasma [ATP] draining skeletal muscle during exercise and hypoxia
Progressive rhythmic handgrip [% of maximal voluntary contraction (MVC)] (A) or single-leg knee extensor (B) exercise significantly increases venous plasma [ATP]. Exposure to systemic hypoxia (inspired O_2 fraction ~10%; hemoglobin O_2 saturation ~80%) results in significantly increased plasma [ATP] as measured by blood sample from deep forearm vein (C) and via intravascular microdialysis in the femoral vein (D) of young healthy humans. Differences in measurement technique contribute to discrepant absolute values between studies (see text for details). $^*P<0.05$ *vs* normoxia/rest; ‡$P<0.05$ *vs* 5% MVC exercise. [Panels A and C adapted from (25). Copyright © 2012 American Heart Association. Used with permission. Panels B and D adapted from (30). Copyright © 2011 The Physiological Society. Used with permission.]

Figure 2. Source of and stimulus for increased venous plasma [ATP] during exercise
Regarding the source of intravascular ATP, two minutes of lower body negative pressure (LBNP) at -40 mmHg to engage the baroreflex and stimulate increased sympathetic nervous system activity did not significantly alter venous plasma [ATP] at rest (A) or during moderate-intensity (15% maximal voluntary contraction) rhythmic handgrip exercise (Ex; B). When forearm blood flow is occluded (Occl) during exercise and muscle contractions continue, ATP is significantly reduced from steady-state exercise (SS Ex) (C). When perfusion is prevented with occlusion prior to exercise, resting [ATP] does not change and exercise fails to increase [ATP] (D). Taken together, these findings suggest the increase in venous plasma [ATP] during exercise arises from cells within or in contact with the blood. n.s. – not significant; $^*P<0.05$ *vs* rest; †$P<0.05$ *vs* steady-state exercise. [Adapted from (22). Copyright © 2013 The Physiological Society. Used with permission.] Regarding the stimulus for ATP release, studies in whole blood samples (E) and plasma measures (F) demonstrate a strong correlation between hemoglobin deoxygenation and increased ATP release. In Panel E, whole blood samples were drawn from Sprague-Dawley rats and then exposed to varying gas concentrations via solution chamber. In Panel F, blood samples were obtained from the human femoral vein at rest and during incremental knee-extensor exercise and plasma was separated. Subjects were exposed to differing systemic atmospheric conditions including normoxia (inspiratory oxygen fraction F_IO_2 21%), hypoxia (F_IO_2 ~10%), hyperoxia (F_IO_2 100%) and carbon monoxide (CO, circulating *F*COHb ~21%) breathing with normoxia in order to vary oxygen saturations. Panel E reprinted from (18). Copyright © 2001 American Physiological Society. Used with permission. Panel F reprinted from (16). Copyright © 2002 American Heart Association. Used with permission.]

Figure 3. Vasomotor responses of intravascular ATP in humans
(A) Intra-arterial (femoral) infusion of exogenous ATP (black line) is capable of causing profound vasodilation (increased vascular conductance), similar to that which occurs with maximal exercise (grey line; incremental cycling exercise). $^*P<0.05$ *vs* rest. [Adapted from (32) Copyright © 2004 The Physiological Society. Used with permission.]. (B) Exogenous ATP is capable of modulating sympathetically-mediated (PE, phenylephrine, α_1-adrenergic agonist) vasoconstriction (decreased vascular conductance), similar to that which occurs during moderate-intensity handgrip exercise (15% maximum voluntary contraction). Here, adenosine

(white bars) is used as a high-flow control vasodilator in quiescent tissue and is not sympatholytic. **P*<0.05 *vs* Pre-PE; †*P*<0.05 *vs* adenosine. [Data from (26) Copyright © 2008 The Physiological Society. Used with permission.].

Figure 4. Similar downstream signaling pathways of exogenous ATP and exercise in humans

(A) Exogenous ATP-mediated vasodilation (increased vascular conductance) in the forearm occurs primarily (~50%) via activation of inwardly-rectifying potassium (K_{IR}) channels as determined via intra-arterial barium chloride ($BaCl_2$) to inhibit K_{IR} channels **P*<0.05 *vs* control. [Reprinted from (3) Copyright © 2012 The Physiological Society. Used with permission.]. Similarly, as seen in panel B, a significant portion (~30%) of the hyperemic response to mild intensity (10% maximal voluntary contraction) rhythmic handgrip exercise is attenuated with infusion of $BaCl_2$, indicating a significant role for K_{IR} channels in this vasomotor response. This inhibition occurs both in the rapid response to muscle contractions at the onset of exercise as well as during steady-state hyperemic conditions. Additional inhibition of Na^+-K^+-ATPase via intra-arterial ouabain does not further impact exercise hyperemia. [Reprinted from (7) Copyright © 2014 American Physiological Society. Used with permission.].

Figure 5. Intravascular ATP as a regulator of vascular tone during conditions of mismatched oxygen supply and demand

Overall schematic of our working hypothesis on the role of ATP in vascular control. (A) In young healthy individuals, during exercise oxygen demand is increased, whereas during hypoxia oxygen supply is diminished. Both instances result in a mismatch that decreases the fraction of oxyhemoglobin (FO_2Hb) and exercise increases CO_2 resulting in acidosis. These conditions, along with exercise-induced mechanical factors and increases in blood temperature serve as stimuli for ATP release from cell sources within or in contact with the blood. Circulating plasma ATP then signals for increased vasodilation and blunts sympathetically-mediated vasoconstriction. Both actions increase red cell supply and therefore oxygen delivery to the tissue in need. The net hyperemic response thus works in a negative feedback manner (red dashed line) to limit the original oxygen deficit and reach a steady-state homeostasis until exercise or hypoxic exposure changes or ceases. (B) In older or diseased populations, failure to adequately increase [ATP] comprises this regulation leading to exacerbated mismatches (positive feedback indicated by solid blue line) in oxygen delivery to demand which can result in relative ischemia.

Figure 6. Older individuals demonstrate impaired vascular control and are unable to increase plasma [ATP] during conditions of oxygen mismatch

Relative to young individuals, older individuals exhibit attenuated hyperemia (A) and fail to increase [ATP] (B) during progressive rhythmic handgrip exercise (% maximal voluntary contraction, %MVC). Similarly, older adults have attenuated hyperemic responses (C) and fail to significantly increase [ATP] (D) in response to systemic hypoxic exposure (inspired O_2 fraction ~10%; hemoglobin O_2 saturation ~80%). **P*<0.05 *vs* rest/normoxia; †*P*<0.05 *vs* young; ‡*P*<0.05 *vs* 5% and 15% within young. [Adapted from (25). Copyright © 2002 American Heart Association. Used with permission.]

Figure 7. Isolated erythrocytes from older and diseased populations fail to release ATP in low oxygen conditions

(A) Isolated erythrocytes obtained from young individuals release ATP upon exposure to low oxygen gas, whereas this does not occur in cells obtained from older individuals. **P*<0.05 *vs* normoxia [partial pressure of oxygen (PO_2) ~110mmHg]; †*P*<0.05 *vs* young. [Reprinted from (25). Copyright © 2012 American Heart Association. Used with permission.]. (B) Similarly, hypoxia-induced ATP release is attenuated in erythrocytes obtained from individuals with type II diabetes mellitus as compared to healthy control subjects. **P*<0.05 *vs* 15% O_2; †*P*<0.05 *vs* 15% and 4.5% O_2. [Adapted from (34) Copyright © 2012 American Physiological Society]. In both studies, tonometry was used to expose isolated erythrocytes to varied O_2 concentrations, 6% CO_2 and N_2 balance.

Figure 1

FIGURE 1.

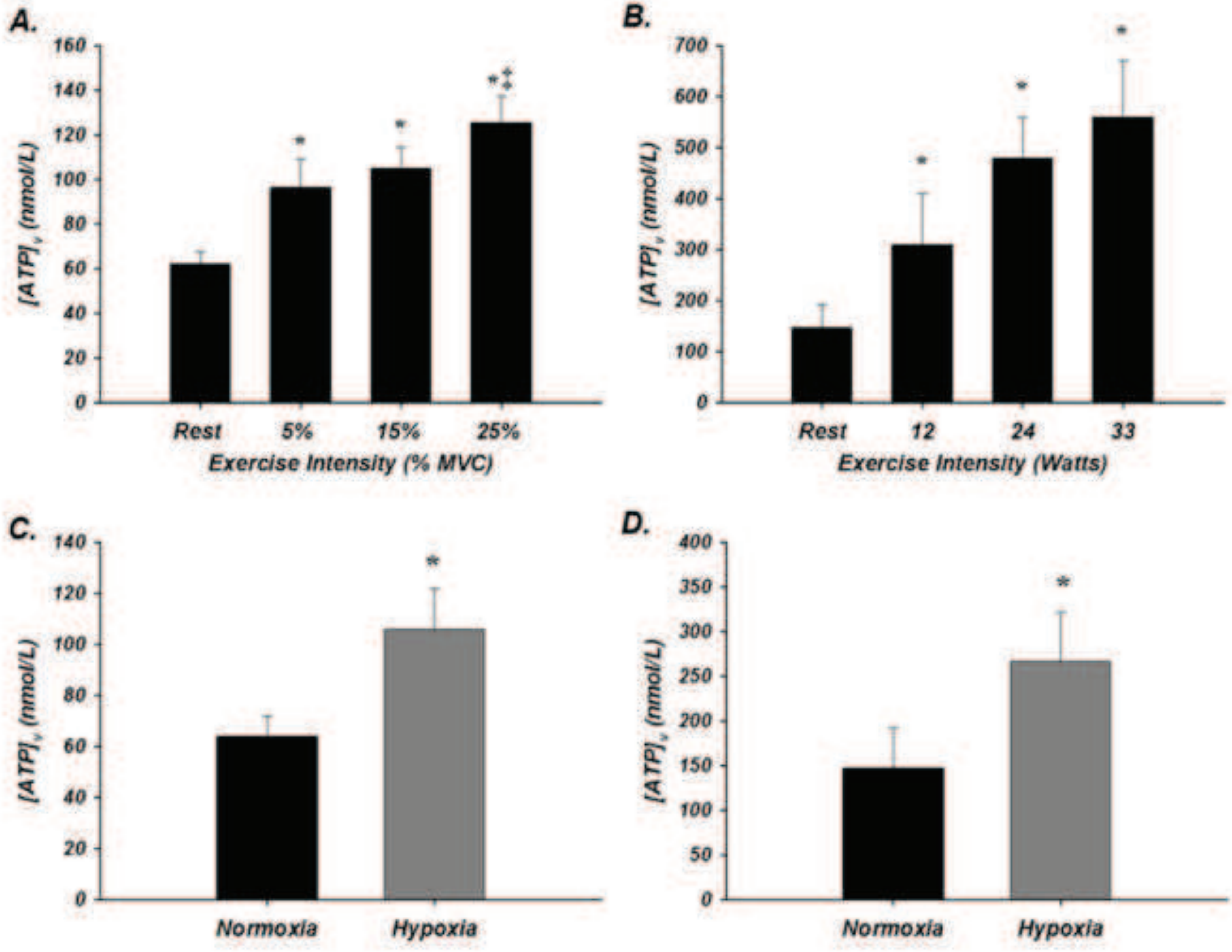


Figure 2

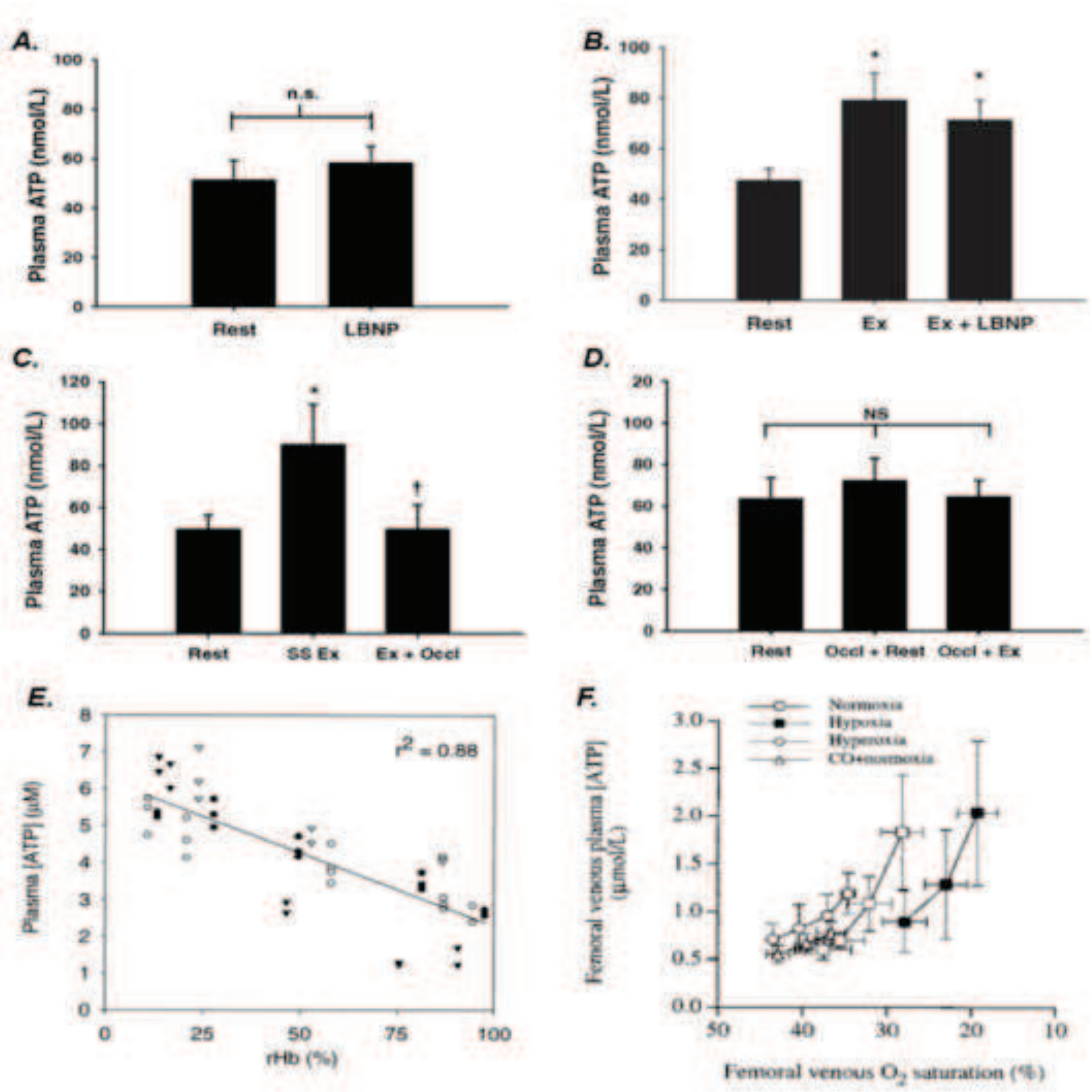

A.
Plasma ATP (nmol/L)
100
80
60
40
20
0
n.s.
Rest
LBNP
B.
Plasma ATP (nmol/L)
100
80
60
40
20
0
*
*
Rest
Ex
Ex + LBNP
C.
Plasma ATP (nmol/L)
120
100
80
60
40
20
0
*
†
Rest
SS Ex
Ex + Occl
D.
Plasma ATP (nmol/L)
20
100
80
60
40
20
0
NS
Rest
Occl + Rest
Occl + Ex
E.
Plasma [ATP] (µM)
8
7
6
5
4
3
2
1
0
$r^2 = 0.88$
0
25
50
75
100
rHb (%)
F.
Normoxia
Hypoxia
Hyperoxia
CO+normoxia
Femoral venous plasma [ATP] (µmol/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
50
40
30
20
10
Femoral venous O_2 saturation (%)

Figure 3

FIGURE 3.

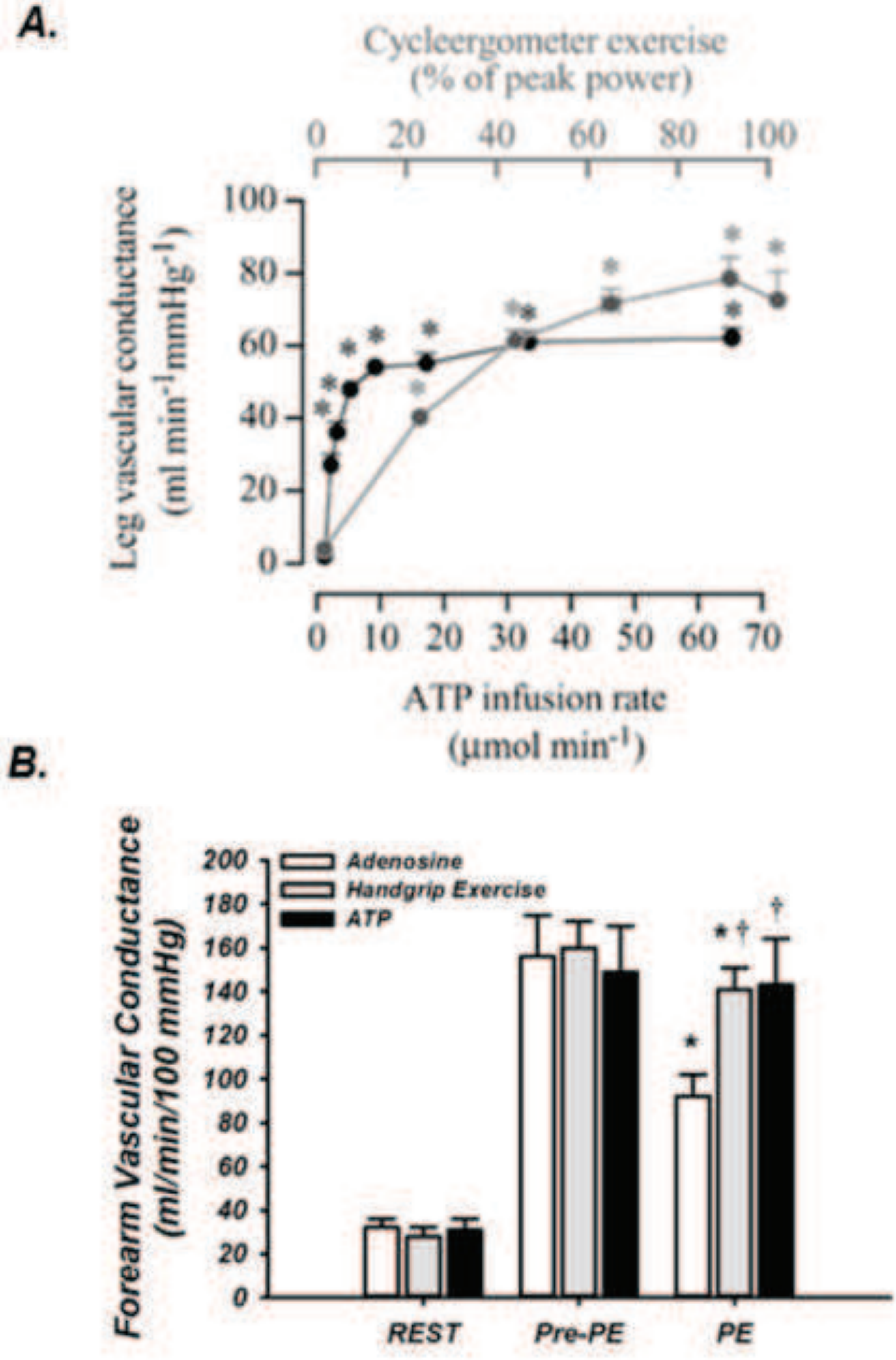


Figure 4

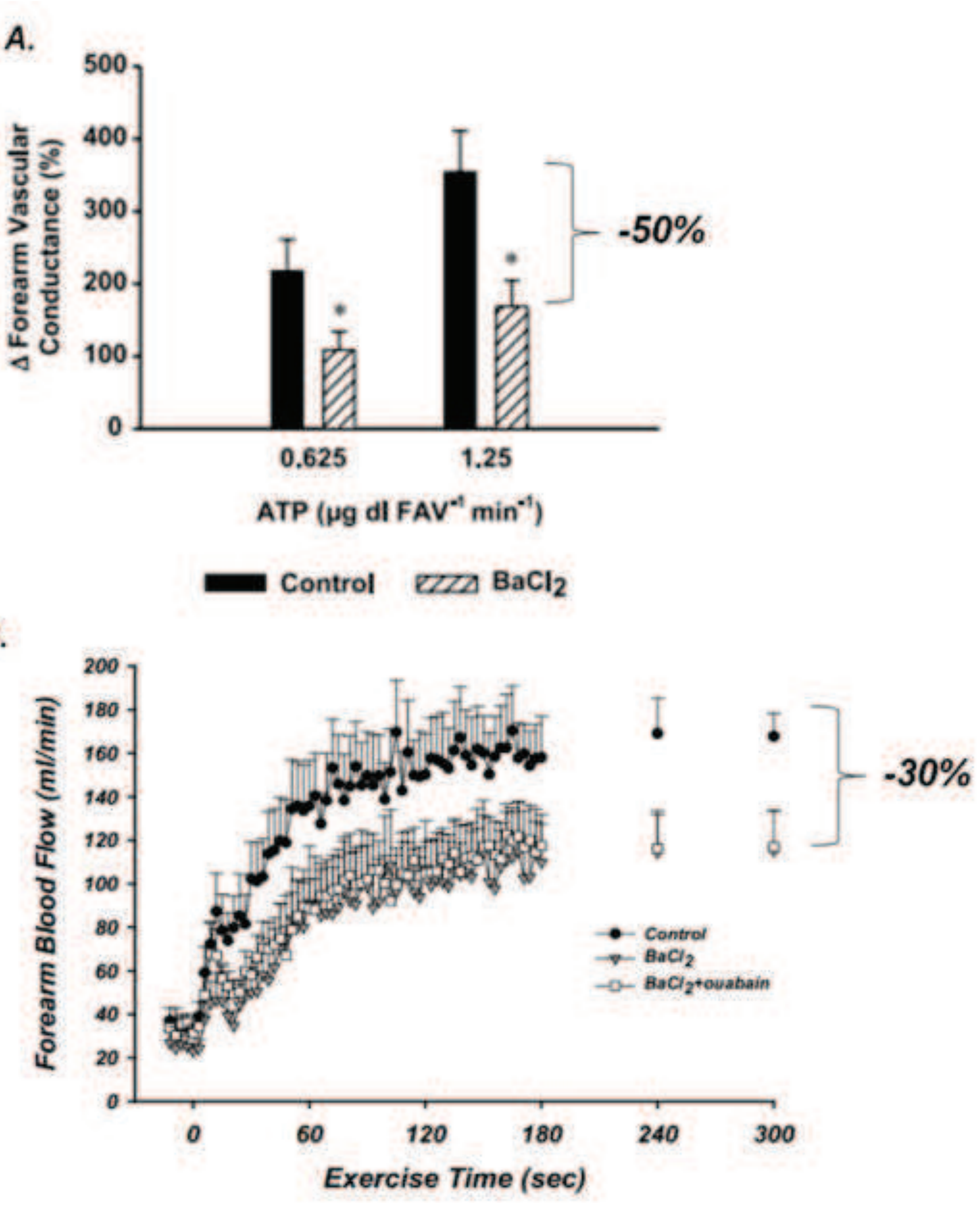

A.
Δ Forearm Vascular Conductance (%)
500
400
300
200
100
0
*
*
-50%
0.625
1.25
ATP (µg dl FAV⁻¹ min⁻¹)
Control
BaCl₂
B.
Forearm Blood Flow (ml/min)
200
180
160
140
120
100
80
60
40
20
0
-30%
Control
BaCl₂
BaCl₂+ouabain
0
60
120
180
240
300
Exercise Time (sec)

Figure 5

FIGURE 5.

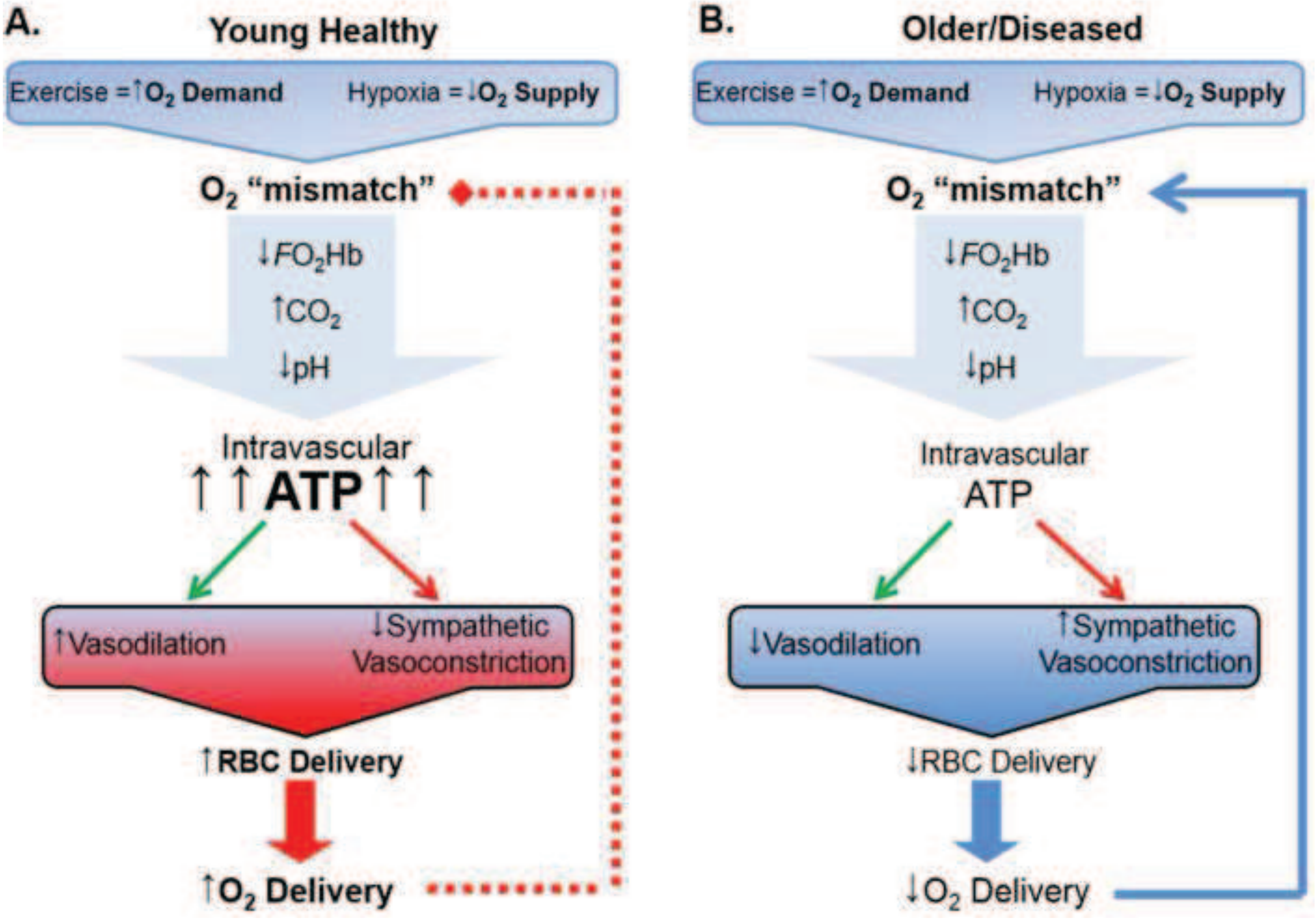


Figure 6

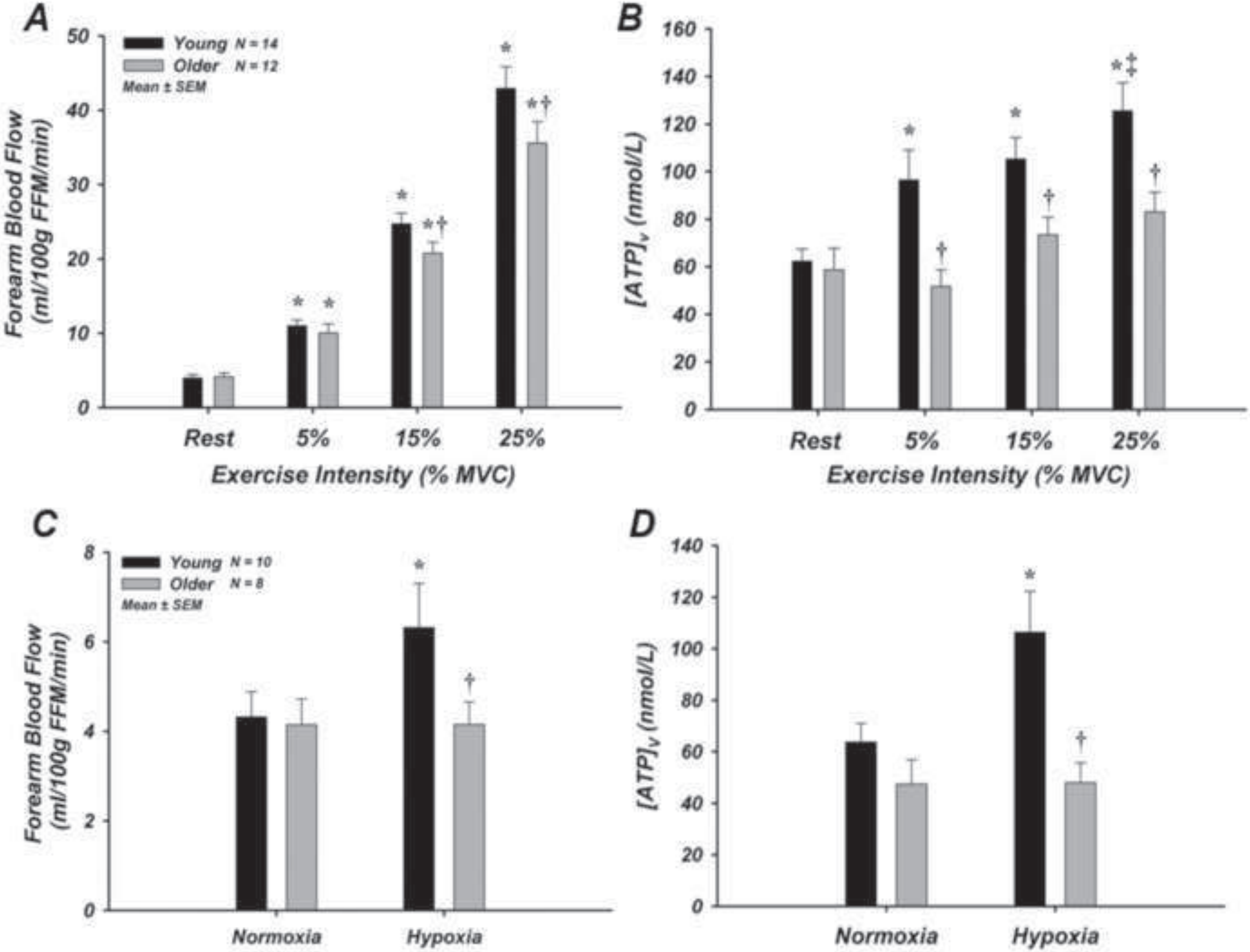
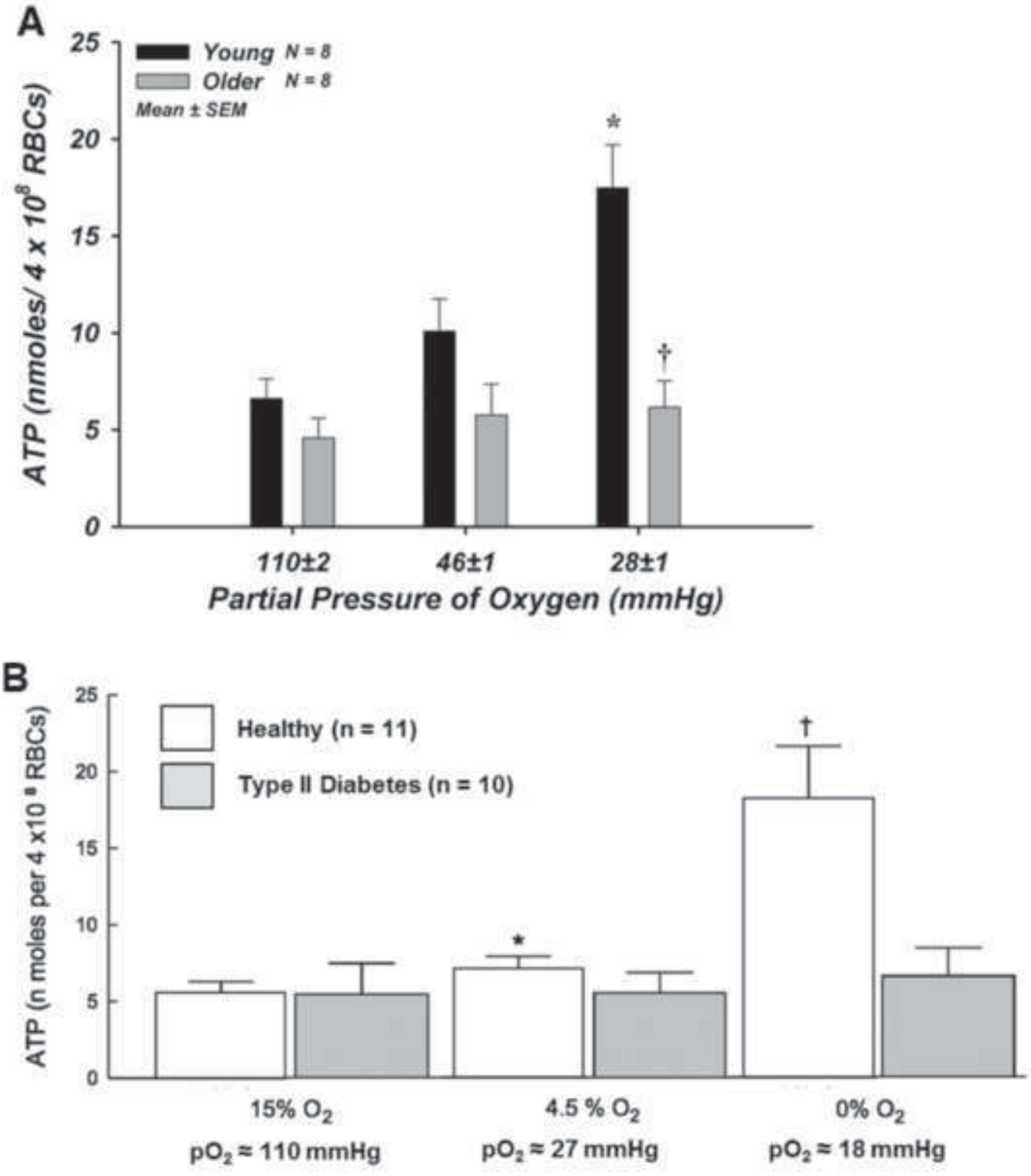

A
Young N = 14
Older N = 12
Mean ± SEM
Forearm Blood Flow (ml/100g FFM/min)
50
40
30
20
10
0
*
*
*
*†
*
*†
Rest
5%
15%
25%
Exercise Intensity (% MVC)
B
[ATP]v (nmol/L)
160
140
120
100
80
60
40
20
0
*
†
*
†
*‡
†
Rest
5%
15%
25%
Exercise Intensity (% MVC)
C
Young N = 10
Older N = 8
Mean ± SEM
Forearm Blood Flow (ml/100g FFM/min)
8
6
4
2
0
*
†
Normoxia
Hypoxia
D
[ATP]v (nmol/L)
140
120
100
80
60
40
20
0
*
†
Normoxia
Hypoxia

FIGURE 7.


A
B
Young N = 8
Older N = 8
Mean ± SEM
ATP (nmoles/ 4 x 10⁸ RBCs)
25
20
15
10
5
0
*
†
110±2
46±1
28±1
Partial Pressure of Oxygen (mmHg)
Healthy (n = 11)
Type II Diabetes (n = 10)
ATP (n moles per 4 x10⁸ RBCs)
15% O₂
pO₂ ≈ 110 mmHg
4.5 % O₂
pO₂ ≈ 27 mmHg
0% O₂
pO₂ ≈ 18 mmHg